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Effect of Bromodeoxyuridine on Endogenous Retrovirus Production in Differentiating Murine Lymphocytes

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A. Introduction

Molecular hybridization studies have revealed the presence of multiple endogenous C-type viruses in germ line DNA of all mouse strains. Expression of viral information results in T-cell leukemias in AKR mice. Study of the control of these genes might be expected to provide information on both leukemogenesis and on gene regulation in eukaryotic cells. In vitro studies of virus induction have shown that bromodeoxyuridine (BrdU) incorporation into fibroblast DNA (Teich et al. 1973) leads to apparent gene derepression and the production of ecotropic and xenotropic viruses (Besmer et al. 1974).

We are interested in the control of endogenous virus expression in lymphocytes, the usual sites of C-type virus-induced diseases. As BrdU incorporation into fibroblast DNA leads to virus induction, the effect of adding BrdU to murine lymphocytes stimulated to proliferate with different mitogens was examined. Since different mitogens stimulate lymphocytes of different cell types, we could examine the effect of BrdU incorporation into different classes of lymphocytes. Surprisingly, we found that it was the target specificity of the mitogen which determined virus inducibility; only B-cells were induced by BrdU, T-cells appearing noninducible (Moroni et al. 1975; Schumann and Moroni 1976). The stimulation of cell proliferation was an absolute requirement for virus induction. In addition, it was observed that B-cell mitogens capable of promoting BrdU induction also induced low levels of xenotropic virus production (Moroni and Schumann 1975). Thus, BrdU appeared to amplify rather than to induce virus, and it remained unclear whether mitogen and BrdU were acting synergistically or in parallel for virus induction. Two approaches to this question are described here. The first involved an examination of the dependence of induction on the stimulation of B-cell differentiation; the second, genetics.

B. Methods

Spleen cell suspensions were cultured for 3 days and assayed for reverse transcriptase and tritiated thymidine incorporation as previously described (Schumann and Moroni 1976). Antibody-secreting cells (ASC) were quantitated by the protein A coated sheep red cell plaque assay of Gronowicz et al. (1976). Xenotropic virus was assayed by the S^+L^- mink F648.1 line (Peebles 1975).

C. Results

It has been reported that terminally differentiated antibody-secreting B-cells express viral antigens (Wecker et al. 1977). Furthermore, all virus-inducing mitogens stimulate the appearance of ASC (Moroni et al., to be published). Thus it could be that ASC produce virus. However, BrdU, which amplifies virus production, inhibits the appearance of ASC. Table 1 shows the effect of BrdU on lipopolysaccharide (LPS) induction of virus in BALB/c mice, either as measured by reverse transcriptase activity or by infectious centers, and on the number of ASC. At a concentration of 5 μg/ml, which is optimal for virus induction, BrdU inhibits the appearance of ASC, without affecting the number of viable cells in LPS-stimulated cultures. These data suggested that virus induction by LPS might depend on LPS stimulation of cell differentiation, whereas

	Reverse transcriptase pmol/2×10⁶ cells	Infectious centers S^+L^-foci/2×10⁶ cells	ASC/2×10⁶ cells
Control	0.04	0	200
LPS	0.42	2	31,800
LPS/BrdU	4.80	220	2,800

Table 1. BrdU amplification of LPS virus induction

	cpm ^{3}H-thymidine incorporated	ASC/2×10⁶ cells	Reverse transcriptase pmol/2×10⁶ cells	
			−BrdU	+BrdU
LPS	45,455	23,000	0.34	4.35
LPS + α-IgM	47,147	3,600	0.24	1.37
+ α-IgM / No α-IgM	1.04	0.16	0.70	0.31

Table 2. Effect of α-IgM on virus induction

virus induction by BrdU would depend on the stimulation of cell proliferation but not differentiation.

It has been shown that addition of an appropriate batch of antisera directed against mouse IgM (α-IgM) to LPS-stimulated cultures blocks the appearance of ASC without affecting cell proliferation (Anderson et al. 1974). Hence, α-IgM was added to LPS and LPS/BrdU treated cultures and reverse transcriptase measured 3 days after stimulation. Table 2 shows that virus production was reduced in both LPS and LPS/BrdU-treated cultures, but only by 30% and 69%, respectively, whereas the number of ASC was reduced by 84%. These results suggest that the stimulation of differentiation is involved in virus induction, at least in the case of LPS/BrdU. Confirmation of this point must await limiting dilution analysis to determine the effect of α-IgM on the number of cells making virus.

In a second approach to investigate B-cell differentiation we used CBA/N mice. They carry an X-chromosome linked, recessive B-cell defect (Cohen et al. 1976). Hence, we compared virus induction in age-matched (CBA/N×BALB/c)F_1 males, which show the CBA/N phenotype, and females (Table 3). Virus induction with both LPS and LPS/BrdU as well as the number of ASC were much lower in males (<25%) than in females. Thymidine incorporation in males was only 50% of the value in females, but we have shown that blocking DNA synthesis to 50% with hydroxyurea only results in a 50% reduction in virus induced by LPS and LPS/BrdU (data not shown). Taken together, the data with α-IgM and CBA/N mice suggest that cell differentiation is required for virus induction with both LPS and LPS/BrdU.

Since lymphocytes from a few strains of mice, e.g., 129, cannot be induced to produce infectious C-type virus, we have also taken a genetic approach to see whether genes for induction with LPS and LPS/BrdU segregate. Induction of xenotropic virus by LPS/BrdU was measured by an infectious center assay of spleen cells on mink S^+L^- cells (Table 1); induction by LPS by cocultivation of stimulated cells with mink CCL-64 cells for several

	cpm ^{3}H-thymidine incorporated	ASC/2×10⁶ cells	Reverse transcriptase pmol/2×10⁶ cells	
			−BrdU	+BrdU
♀	10,266	28,800	1.04	5.88
♂	5,224	4,200	0.24	0.47
♂/♀	0.51	0.15	0.23	0.08

Table 3. Induction in (CBA/N×BALB/c)F_1 mice

passages (Monckton and Moroni 1980) followed by assaying for infectious xenotropic virus. BALB/c×129 crosses were kindly performed for us by Ms. Hämmerli (Tierfarm Sisseln). All BALB/c mice tested (15/15) were positive by these procedures, while all 129 mice (10/10) were negative. All (BALB/c×129)F_1 mice resembled BALB/c, i.e., inducibility was dominant. F_1 mice were backcrossed to 129 mice and tested for inducibility by LPS/BrdU; of these, 32/60 (53%) showed the BALB/c phenotype, implying that one gene controls induction by LPS/BrdU. Spleen cells from a number of the same mice were also tested for LPS induction. A perfect correlation was observed between LPS/BrdU and LPS: 17/17 LPS/BrdU positive mice were positive with LPS alone and 15/15 LPS/BrdU negative mice were noninducible with LPS. Induction with LPS and LPS/BrdU seemed, therefore, under the control of the same gene.

D. Discussion

LPS induction and BrdU "amplification" of endogenous virus production in BALB/c lymphocytes have a number of features in common. In both cases xenotropic virus is produced. Induction is controlled by the same genetic locus, probably the structural gene for BALB virus-2. The finding that BrdU increases the number of infectious centers by a factor of ten more than reverse transcriptase (Table 1) might well reflect an increased virus production per cell and hence an increased probability of detection. Both processes appear to require cell proliferation and differentiation. This suggests that murine lymphocytes release xenotropic virus at a certain stage along a normal differentiation pathway and that B-cell mitogens induce virus by stimulating the appearance of cells which produce virus. Antibody secretion itself does not appear to be required for virus production, since BrdU amplifies virus while at the same time inhibiting ASC.

Incorporation of BrdU into the DNA of three different types of cells derived from BALB/c mice has three different consequences for endogenous virus expression. In fibroblasts xenotropic and ecotropic viruses are induced, in stimulated B-cells xenotropic virus production is amplified, and in stimulated T-cells no infectious virus production occurs. The observation that α-IgM reduces the level of LPS/BrdU induction from B-cells suggests that certain differentiated functions, acquired only late in B-cell differentiation and present in fibroblasts, are required for virus induction. One class of candidates for this differentiation function are RNA-processing enzymes. The switch from surface to secreted IgM, which takes place after LPS stimulation, is thought to involve an alteration in mRNA processing (Singer et al. 1980). In a manner analogous to the failure of C-type viruses and SV40 to replicate in undifferentiated teratoma cells (Teich et al. 1977; Segal et al. 1979), the absence of the appropriate processing enzymes in T-cells might render them refractory to induction by BrdU.

References

Anderson J, Bullock WW, Melchers F (1974) Eur J Immunol 4:715 – Besmer P, Smotkin D, Haseltine W, Far H, Wilson AT, Paskin JM, Weinberg R, Baltimore D (1974) Cold Spring Harbor Symp Quant Bid 39:1103 – Cohen PL, Scher I, Mosier DE (1976) J Immunol 116:301 – Gronowicz E, Coutinho A, Melchers F (1976) Eur J Immunol 6:588 – Monckton RP, Moroni C (1980) J Gen Virol 47:59 – Moroni C, Schumann G (1975) Nature 254:60 – Moroni C, Schumann G, Robert-Guroff M, Suter ER, Martin D (1975) Proc Natl Acad Sci USA 72:535 – Moroni C, Stoye JP, DeLamarter JF, Erb P, Jay FA, Jongstra J, Martin D, Schumann G (1980) Cold Spring Harbor Symp Quant Biol 44:1205 – Peebles PT (1975) Virology 67:288 – Schumann G, Moroni C (1976) J Immunol 116:1145 – Segal S, Levine AJ, Khoury G (1979) Nature 280:335 – Singer PA, Singer HH, Williamson AR (1980) Nature 285:294 – Teich N, Lowy D, Hartley JW, Rowe WP (1973) Virology 51:163 – Teich NM, Weiss R, Martin GR, Lowy DR (1977) Cell 12:973 – Wecker E, Schimpl A, Hünig T (1977) Nature 269:598